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Storm and *Ips typographus* disturbance effects on carbon stocks, humus layer carbon fractions and microbial community composition in boreal *Picea abies* stands

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ABSTRACT

Tree-killing forest disturbances such as storms and bark beetle outbreaks can lead to notable changes in the carbon (C) balance and functioning of forest ecosystems. In this study, the effects of a storm in 2010 followed by an outbreak of European spruce bark beetle (*Ips typographus* L.) on tree, litter and soil C stocks as well as humus layer C fractions and microbial community composition were examined in boreal Norway spruce (*Picea abies* L.) stands. Tree (aboveground), litter detritus (distinguishable twig, bark and cones) and soil (humus layer and 0–6 cm mineral soil) C stocks were quantified for undisturbed (living trees), storm disturbed (in 2010) and *I. typographus* disturbed (tree mortality in circa 2013–2014) plots in 2015–2016. Additional humus layer samples were collected in 2017 for determination of total microbial biomass C, ergosterol (fungal biomass indicator) and K_2SO_4 extractable (labile) C concentrations, as well as fungal and bacterial community composition (DNA sequencing). Ectomycorrhizal (ECM) fungal mycelial growth in topsoil was also quantified. In spite of the differing initial development and intensity of the two disturbance types, there was little difference in humus layer C and microbiology between the storm and bark beetle disturbed plot types at the time of the study. This may be due to the longer time since the disturbance at the storm disturbed plots. The shift from tree biomass to necromass C stocks was not reflected in differences in SOC stocks or humus layer extractable C concentrations between undisturbed and disturbed plot types, but the amount of litter detritus on forest floor was similar (storm) or higher (beetle) in disturbed plots in comparison to undisturbed ones. Humus layer microbial biomass C and ergosterol concentrations and ECM fungal abundance were lower on disturbed plots in comparison to undisturbed plots. The disturbed plots were also indicated to have a slightly higher abundance of some saprotrophic fungi. Differences in the effects of the two disturbance types may occur when studied at differing spatial scales and at different times after disturbance. To understand the full impact of such disturbances on forest functioning and C balance, long-term monitoring studies will be required.

1. Introduction

Disturbances are known to be essential drivers of forest ecosystem composition and functioning (Edburg et al., 2012; Mitchell, 2013; Ulanova, 2000). The predicted increase in tree damage and mortality by various natural forest disturbances and their interactions (Seidl et al., 2017; Seidl and Rammer, 2017) has, however, raised concerns about the effects of disturbance on forest carbon (C) stocks and fluxes. Decreased gross primary production and changes in ecosystem respiration resulting from severe disturbance and tree mortality can notably reduce forest C sequestration and temporarily turn a forest from being a C sink into a C source (Ghimire et al., 2015; Hicke et al., 2012; Kurz et al., 2008; Lindroth et al., 2009). Depending on the amount of remaining living trees and stand structure, the effects can, however, be transient and less severe (Brown et al., 2010; Reed et al., 2014).

Storms and outbreaks of the European spruce bark beetle (*Ips typographus* L.) are two major natural disturbances of forests in Europe, and tree damage and mortality by both are expected to increase in the

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future (Bentz et al., 2019; Seidl et al., 2017). However, the nature of these two disturbances differ. Storms have an immediate impact, with uprooted and broken trees usually dying soon after the event and is often associated with disturbance of the soil. Tree mortality by *I. typographus* and vectored pathogenic fungi develops more gradually as the flow of photosynthates in the host-tree phloem is hindered (Biedermann et al., 2019), and may result in more patchy and spatially interspersed infestation and tree mortality patterns (Kärvemo et al., 2014b; Økland et al., 2016) than severe storms.

Although the dynamics of tree mortality differ, storm and bark beetle disturbance may be expected to have similar effects on soil functioning and C cycling. In addition to tree belowground allocation of photosynthates, litter inputs to soil (Bradford et al., 2012; Kopáček et al., 2015; Trahan et al., 2015) would be affected. For example, litterfall from trees killed by bark beetles is often massive shortly after the outbreak (Kopáček et al., 2015). Also, soil microclimate (Mayer et al., 2017, 2014; Morehouse et al., 2008), microbial community composition and functioning (Šimonovičová et al., 2019; Štursová et al., 2014), as well as decomposition and nutrient cycling (Cigan et al., 2015; Mayer et al., 2017; Sariyildiz et al., 2008) are often altered by the tree mortality and related changes in the environment. Such changes and their magnitude can be expected to change over time and to vary depending on disturbance intensity (tree mortality) and whether the dead trees are harvested afterwards.

Wind and harvest disturbances have been shown to affect forest floor (soil organic layers) C stocks already during the first decade after the event, whereas changes in mineral soil C stocks are often less or at least are slower (Bradford et al., 2012; James and Harrison, 2016; Mayer et al., 2017; Nave et al., 2010; Piirainen et al., 2015). However, soil labile C fractions and microbial community composition often respond quickly to disturbance. Tree death may result in a decrease in the abundance and richness of tree-symbiotic ectomycorrhizal (ECM) fungi (Mayer et al., 2017; Treu et al., 2014; Pec et al., 2017; Štursová et al., 2014), whereas saprotrophic fungi can benefit from the increase in the supply of dead organic matter soon after disturbance (Štursová et al., 2014; Treu et al., 2014). Depending on the changes in belowground allocation of photosynthates and litter inputs and other changes in the environment, microbial biomass and/or dissolved organic C (DOC) concentrations can at least temporarily decline during the first years after storm and bark beetle disturbance (Gömöryová et al., 2011; Štursová et al., 2014; Trahan et al., 2015), whereas temporary increases during the first years after disturbance have also been shown (Kaňa et al., 2015).

In this study we investigated the effects of storm and *I. typographus* induced disturbances on tree and soil C stocks, humus layer C fractions and microbial community composition. The C stocks (tree aboveground, litter detritus, humus layer, and top mineral soil), and humus layer labile C fractions and various measures of microbial abundance and diversity (K_2SO_4 extractable and microbial biomass C, fungal biomass, ECM fungal mycelial growth, fungal and bacterial community composition) of storm and *I. typographus* disturbed plots were quantified and compared with those from undisturbed (living trees) plots. The study took place five to seven years after the storm and about one to four years after major tree mortality caused by *I. typographus*.

Given the differences in the nature and dynamics of tree mortality between storm disturbances and bark beetle outbreaks described above, and the difference in the time since the two disturbances occurred at the study area, we hypothesized that: 1) litter detritus (distinguishable twigs, bark and cones) C stocks would be greater at the disturbed plots than at the undisturbed plots, but C stocks of the humus layer and top mineral soil between the plot types would not differ, and 2) ECM fungal abundance and humus layer K_2SO_4 extractable (labile) C, microbial biomass C and fungal biomass concentrations would be lower in the disturbed plot types, most distinctly at the storm affected plots. These microbiological differences between the plot types were also expected to be reflected in the differences in bacterial and fungal communities indicated by DNA sequencing.

2. Materials and methods

2.1. Study area and plot establishment

The study was carried out in two Norway spruce (*Picea abies* L.), dominated forest sites, Paajasensalo (56 ha) and Viitalampi (73 ha), located near each other (circa 6 km apart), in Ruokolahti (61°17'30''N, 28°49'0''E), southeastern Finland. A severe storm hit the area in summer 2010 and was followed by an outbreak of *I. typographus* from 2011 onwards. Such interaction between the two disturbance types is typical as wind-fallen trees provide optimal breeding material for *I. typographus* (Kärvemo et al., 2014a). Tree mortality due the beetle mostly occurred in 2014–2015. Prior to the storm, the sites were under forest management, but gained conservation status after the storm, and thus the trees killed by the disturbances have been left on site.

The soils at both sites were mainly cambic podzols developed in till deposits with a sandy loam or loamy sand soil texture and a moder type humus layer. In addition to *P. abies*, Scots pine (*Pinus sylvesteris* L.) and deciduous trees (e.g. *Betula pubescens* L., *Populus tremula* L., *Betula pendula* Roth, *Alnus incana* L., *Alnus glutinosa* L. Gaertn., *Sorbus aucuparia* L.) grow in these mature forests. According to Cajander's site type classification (Cajander, 1949; Mikola, 1982), the forest site types in the area are mostly medium-rich (MT, Myrtillus type) and herb-rich fertile (OMT, Oxalis-Myrtillus) types with ground vegetation being dominated by mosses (mainly *Pleurozium schreberi* (Brid.) Mitt., *Hylocomium splendens* (Hedw.) BSG and *Dicranum* spp.), shrubs (*Vaccinium myrtillus* L. and occasional *Vaccinium vitis-idaea* L.), and occasional herbaceous plants (e.g. *Oxalis acetocella* L., *Melampyrum sylvaticum* L., *Linnea borealis* L.). However, the disturbances, especially the storm, had modified the ground vegetation composition in the more open areas towards more of light-demanding pioneer species (e.g. *Rubus ideaus* L., *Epilobium angustifolium* L.) and grasses (e.g. *Deschampsia flexuosa* L.Trin). For period 1981–2010, the mean annual precipitation in the study area was 653 mm and temperature 4.2 °C (Pirinen et al., 2012).

Areas representing three types of stands (hereafter referred to as plot types) were identified in the two sites: living trees with no clear disturbance (LT), storm-felled trees (SF), and *P. abies* trees killed by *I. typographus* (ID). Circular plots (400 m^2), representing each of the three plot types were then established in the summer of 2015 (6 plots) and in the summer of 2016 (6 plots). Although some of the dead trees at the SF plots had been infested by *I. typographus*, the initial cause of death was the storm in 2010. The dead *P. abies* trees on the ID plots had been infested by *I. typographus* from 2011 onwards and tree mortality was estimated to have occurred in 2013–2014. Other bark beetles, such as the sixtoothed bark beetle (*Pityogenes chalcographus* L.), typically occurring concurrently with *I. typographus* outbreaks (Göthlin et al., 2000), were also detected and probably were the cause of death of some smaller *P. abies* trees at the ID plots. Some of the *P. abies* tree trunks on the LT plots had visible entrance holes of *I. typographus*, but the trees remained vigorous during our study. A more detailed description of the sites and plot set-up, along with photographs, can be found in Kosunen et al. (2019).

2.2. Field work and sampling

2.2.1. Sampling for determination of C stocks

The trees were measured and samples of litter detritus, the humus layer and underlying mineral soil collected from each plot in August 2015 (2015 established plots) and in 2016 (2016 established plots) for determination of above- and belowground C stocks. The diameter at breast height (dbh) of each living and dead tree with a dbh more than 6 cm was measured. The height of those trees was also measured when possible (74% of trees). If a trunk was broken, the height of the standing and, if found, fallen parts were measured separately, otherwise only the

dbh of the standing part was recorded. Samples of litter detritus, humus layer and underlying (0–6 cm) mineral soil were collected from 12 sampling points per plot (at 2.8 m outwards from plot center in each cardinal direction and at 8.5 m in each cardinal and half-cardinal direction). If a sampling point was not representative (e.g. stone, tree, stump, pit created by windfalls with no humus layer), it was moved to the nearest suitable spot. At each sampling point, the ground vegetation except for mosses (if any) was first removed and the clearly distinguishable pieces of litter detritus (twigs <1 cm diameter, bark and cones) were collected from a 0.04 m^2 area. More decomposed (not easily distinguishable to origin) litter was removed before a 0.01 m^2 humus layer ($O_f + O_h$) sample was cut and its thickness recorded. The surface of the mineral soil was then exposed and a sample taken using a steel cylinder (surface area = 26.4 cm^2, height = 5.9 cm). After returning from field, the samples, each in separate plastic bags, were stored in −20 °C.

2.2.2. Sampling for determination of humus layer C fractions, microbial abundance and community composition

To estimate the growth of ectomycorrhizal (ECM) fungal mycelium, the in-growth mesh bag method (Wallander et al., 2001) was used. The in-growth bags (mesh size 50 μm), containing 120 g of acid-washed quartz sand (0.5–1.5 mm, SP Minerals), were inserted into the soil at seven points per plot (next to the plot center, 5.6 m outwards from plot center in each cardinal direction and at 9.0 m in North and South directions) in June 2017. If a sampling point was not representative (e.g. stone, tree, stump), it was rejected, and the nearest suitable point taken. A steel auger was used to drill a vertical hole and the bags inserted so that the top was located at the interface of litter and humus layers. The in-growth bags were retrieved in November 2017 (i.e. after circa 22 weeks), placed into plastic bags and into a cooler box for transport to the laboratory where they were stored at +4 °C until analyzed.

For the determination of C fractions, microbial abundance and community composition another set of humus layer samples was collected in August 2017. The samples were taken adjacent (circa 0.8 m) to each in-growth bag following the same procedure as in 2015 and 2016, but the samples were placed immediately into a cooler box to be transported to the laboratory, where they were first stored at +4 °C during sample pre-handing.

2.3. Calculation of C stocks

2.3.1. Aboveground tree C stocks

Aboveground tree mass (tree stem, branches plus foliage) was calculated using the biomass equations developed by Marklund (1988) for *P. sylvestris, P. abies* and birch (*P. pendula* and *pubescens*). The equation for birch was applied to all deciduous trees (78% of which were birch). These functions use over-bark dbh alone or together with tree height to estimate aboveground tree dry weight. For dead *P. abies* trees that had lost their bark, bark thickness was estimated using a previous data set on bark thickness for similar sized *P. abies* trees in the area (data not shown), and added to the measured dbh of the tree. A dry weight C concentration of 50%, which often has been observed for coniferous tree stems, was used for calculating tree C stocks (Ma et al., 2018; Sandström et al., 2007). Tree aboveground necromass C stock was corrected for density loss by decay using annual decay rate constants of 3.3% for pine, 3.4% for spruce and 4.5% for birch (used for all deciduous trees) (Krankina and Harmon, 1995), and the number of years since tree death in 2016 to be six years for SF plots and two years for ID plots. For trees that had died prior to the storm in 2010 (cause unknown), the number of years since death was taken to be 10 years. Since the forests had been in commercial use before 2010, the number of such trees was small (7% of all trees).

2.3.2. Litter detritus, humus layer and soil C stocks

Litter detritus, humus layer and mineral soil samples (n = 48 per plot type) collected in 2015 and 2016 were dried (50 °C, 96 h). Twigs (<1 cm diameter), bark and cones in the litter detritus samples were separated, weighed, and milled to a fine powder. The humus layer samples were weighed, roots picked out and weighed, and the remaining material milled. The mineral soil samples were sieved (2 mm mesh size) and weight of the >2 mm and <2 mm fractions, as well as that of the roots, recorded. Total C concentrations were determined with a VarioMax CN-device (Elementar Analysensysteme GmbH, Hanau, Germany).

Litter detritus C stocks were calculated from the dry weights, sample area and C concentrations for twigs, bark and cones. Humus layer and mineral soil (<2 mm) C stocks, including that of the roots, were calculated using the dry weight bulk density of each sample, sampled layer thickness, and measured C concentrations, and an assumed C concentration of 50% for roots.

2.4. Humus layer C fractions and microbial community composition

2.4.1. Sample pre-handling

In the laboratory (2–5 days after collection), the humus layer samples from 2017 (n = 28 per plot type) were homogenized by removing roots and litter fragments. For DNA sequencing, subsamples (ca. 5 ml) of each homogenized sample were composited by plot type and study site to produce six samples altogether, i.e. two samples per plot type (Supplementary material 1). For the fumigation extraction, ergosterol and total C analysis, the samples were composited so as to produce three samples per plot (i.e. two composite samples made from two samples and one composite sample made form three samples; selection of samples to composite being random), i.e. 12 samples per plot type. The in-growth bags (n = 28 per plot type) were not composited. Sample composition and storage condition details are summarized in Supplementary material 1.

2.4.2. Microbial biomass, soil extractable and total C

The chloroform fumigation-extraction method (Vance et al., 1987) was used to estimate microbial biomass C (C_{MB}) from the humus layer samples. Briefly, after fumigation of subsamples with chloroform, extraction was done with a 0.05 M potassium sulphate solution (K2SO4; 1:20). Another set of subsamples were handled in the same way, but not fumigated and taken straight to extraction. TOC concentrations in the filtrates were determined using a total organic C (TOC) analyzer (Shimadzu TOC-V CPH, Shimadzu Corp., Kyoto, Japan). C_{MB} concentration (mg g^{-1} of sample dry weight) was calculated as the difference between fumigated and non-fumigated C concentrations divided by 0.45 (Vance et al., 1987). Non-fumigated K_2SO_4 extractable C corresponds to other labile forms of soil organic C (C_{EXT}) (Makarov et al., 2013). Total C concentrations were determined from dried (50 °C, 48 h) subsamples with VarioMax CN-device.

2.4.3. Ergosterol analysis

The concentration of ergosterol, a biomarker indicator of fungal biomass, was determined from the humus layer samples using high-performance liquid chromatography (HPLC) (Frostegård and Bååth, 1996) as described in Adamczyk et al. (2019b). Briefly, ergosterol was extracted with cyclohexane and 10% KOH in methanol. Cyclohexane phase was removed, evaporated and residue was dissolved in methanol. The amount of ergosterol was measured with HPLC (HP Agilent 1100, Hewlett Packard, USA) using a C18 100A reverse-phase column. As a standard, pure ergosterol (Sigma-Aldrich, cat no 45480) was used. Concentrations of ergosterol were calculated per sample dry weight.

2.4.4. Ectomycorrhizal fungal growth

Each in-growth bag was cut open and the contents mixed. Sand from each bag was viewed under a stereomicroscope and the abundance class of ECM fungal hyphae assessed (0 = no hyphae, 1 = even one visible hyphae, 2 = some hyphae easily found and slight aggregation of sand, 3 = several hyphae easily found and clear aggregation of sand).

Subsamples were then taken to determine ergosterol concentration using the same procedure as described above. To obtain an index describing the ectomycorrhizal mycelial abundance and growth (ECM_{growth}), the visual abundance estimates and ergosterol concentrations were normalized, summed and then normalized again, following Mayer et al. (2017).

2.4.5. DNA extraction, MiSeq sequencing, processing and analysis of sequence data

The plot type samples for DNA sequencing were thawed and DNA extracted using NucleoSpin for soil kit (Macherey Nagel, Germany) according to the manufacturer's protocol and DNA concentrations were measured with Nanodrop One (Thermo Scientific) and sequenced at the Institute of Genomics, the Tartu University, Estonia. For bacteria, the targeted V4 region of the 16S SSU rRNA and the ITS2 region for fungi were amplified in a two-step polymerase chain reaction (PCR) using the 16S rRNA primers 515F and 806R (Caporaso et al., 2012, 2011) and ITS4 (White et al., 1990) and gITS7 (Ihrmark et al., 2012) with 8bp dual index for 24 cycles. The final PCR fragments were run as paired-end 2 × 300 bp with the MiSeq platform (Illumina) using MiSeq v3 kit producing about 20–25M reads per flowcell.

Quality filtering, removal of artifacts, primer-dimers and primers from raw 16S rRNA and ITS sequence reads was conducted with the PipeCraft 1.0 pipeline software (Anslan et al., 2017). PipeCraft utilizes several implemented tools, e.g., mothur v1.36.1 (Schloss et al., 2009), vsearch v1.11.1, CD-HIT v4.6 (Fu et al., 2012) and swarm v2.1.8 (Mahé et al., 2015) that are used in pre-processing, assembling, chimera filtering and clustering steps. Briefly, assembly of paired end reads and initial quality filtering was conducted with vsearch (v1.11.1; github.com/torognes/vsearch; Rognes et al., 2016) with minimum overlap 15, max differences 99, minimum length 150bp, e_max 1, max ambiguous 0 and trunc qual 10 and 20 for bacteria and fungi, respectively. On average 35% of the raw reads were filtered out after the assembly. Chimera filtering was performed for the reoriented reads by using vsearch (v1.11.1; github.com/torognes/vsearch) de novo filtering with parameters: annotation 0.97 and abskew 2; and for ITS both reference based filtering was used with Unite ITS2 ref v7.1 as data base. The fungal ITS2 region was extracted from reads with ITSx (Bengtsson-Palme et al., 2013). Sequence reads were then clustered and an OTU table created with CD-hit (Fu et al., 2012) with parameters: threshold 0.97 and min size 2. Finally, the OTUs were taxonomically annotated by searching representative bacterial or fungal sequences with BLAST using reference 16S rRNA (SILVA_123_SSURef_Nr99_tax_silva.fasta) or ITS2 databases (sh_genral_release_dynamic_December 01, 2018.fasta) obtained from SILVA (Quast et al., 2013; Yilmaz et al., 2014) and UNITE (Nilsson et al., 2018) respectively. Based on the BLAST results OTUs that had an e-value higher than e−25, query coverage less than 90% and identity less than 70% with the database match were filtered out. OTUs that had affiliation other than to bacteria or fungi and all OTUs of relative proportion below 0.0001% were also removed from the data (<13 reads). FUNGuild was used to detect functional information on fungal guilds of OTUs (Nguyen et al., 2016). A total of 136 171 reads were retained to make 462 OTUs representing fungal community, and 116 044 reads to make 549 OTUs representing bacterial community. The raw sequence data was deposited to the sequence read archive (SRA) of NCBI/EMBL databases with the accession number PRJNA575623.

2.5. Statistical analyses and visualization of data

Significant differences in the litter detritus and soil properties between the plot types were tested using an ANOVA having a linear mixed effects model structure, followed by Tukey's pairwise comparisons of the estimated marginal means. In the mixed effects model, plot type and interaction between plot type and forest site (Paajasensalo and Viitalampi) were fixed factors and plot number (1–12) a random factor accounting for potential spatial autocorrelation of samples collected from the same plots. If interaction between forest site and plot type or forest site did not show a significant effect in the model, they were removed, and only plot type left as a fixed factor. This was the case for all quantified variables except humus layer ergosterol concentration. Transformations (square root or cube root) were applied if needed. Differences in aboveground living and dead tree C stocks between the plot types were not tested statistically as they were plot-level variables (n = 4 per plot type). The DNA sequenced microbial group-level data was not statistically tested due to the low number of observations (n = 2 per plot type), but principal components analysis (PCA) was conducted to visualize the differences in microbial community composition between the plot types. Also, shared and unique OTUs between plot types were visualized using Venn diagrams, and proportions of most dominant fungi and bacteria at each plot type were visualized with heatmaps. Statistical testing and visualization of the data with Venn diagrams was carried out using the R-statistical computing environment (R Core Team, 2019). Package lme4 (Bates et al., 2015) was used for linear mixed modeling, car (Fox and Weisberg, 2019) for ANOVA and emmeans (Lenth, 2019) for post-hoc testing. Packages venn (Chen et al., 2018) and VennDiagram (Dusa, 2018) were used to create Venn diagrams. Rarefaction curves (Supplementary material 7) were conducted for OTU sequence data using the vegan package (Oksanen et al., 2019) to verify that OTU data in all sample libraries were comparable to be used in PCA to visualize the difference between sample types. PCA was conducted using Canoco 5 for Windows (Microcomputer Power, Ithaca, NY, USA, 2012).

3. Results

3.1. Stand characteristics of the plots

Plot mean dbh of all (living and dead) trees varied between 14.7 and 24.8 cm, and plot mean tree height between 12.4 and 21.5 m (Table 1). The living tree basal area among the disturbed plots ranged between 0 and 22.8 m^2 ha^{-1} and that of dead trees between 20.1 and 39.7 m^2 ha^{-1} (Table 1). The living to all tree basal area rations varied between 0.81 and 0.99 at the LT plots, 0.00 and 0.36 at the SF plots and 0.07 and 0.36 at the ID plots, the SF plots in Viitalampi site having the lowest ratios. Among all plots, proportion of *P. abies* covered 45–100% of all (living or dead) trees (Table 1), however, on the plot with the lowest proportions (Viitalampi LT plot established in 2016), 59% of the living trees were spruce.

3.2. Tree, litter detritus, humus layer and soil C stocks

Aboveground mean tree C stock averaged by plot type were 116 Mg C ha^{-1} for LT, 78 Mg C ha^{-1} for SF, and 108 Mg C ha^{-1} for ID (Fig. 1a). The mean aboveground total tree C stocks of the disturbed plots was however slightly lower (not statistically tested) than that of the LT plots already without the decay corrections (120 for the LT, 93 for the SF and 114 for the ID plots). The proportion of dead tree necromass per all tree C stocks was 7% for LT plots, 80% for SF plots and 71% for ID plots. The estimated mean change in the aboveground tree necromass C stocks due to the decay loss six years after the storm at the SF plots and two years after *I. typographus* caused tree mortality at the ID plots, was 19% (14.9 Mg C ha^{-1}) and 7% (5.5 Mg C ha^{-1}), respectively.

Litter detritus (distinguishable twigs <1 cm in diameter, bark and cones) C stocks were significantly and more than twice higher on the ID plots in comparison to LT and SF, the difference mostly owing to the greater amount of bark and cones on the ID plots (Fig. 1b). Humus layer or mineral soil (0–6 cm depth) C stocks did not differ between the plot types (Fig. 1c).

Table 1
Plot stand characteristics at the Paajasensalo (PS) and Viitalampi (VL) study sites. LT = Undisturbed, living trees, SF = Storm killed trees, ID = *I. typographus* killed trees.

Study site	Plot type	Year of plot establishment	dbh, cm [a]	h, m [b]	Number of stems per ha[c]				Basal area, m^2 ha^{-1}			
					Total	S%	P%	D%	Living	Dead	Total	Living/Total
PS	LT	2015	20.6	18.6	1350	65	22	13	42.2	9.3	51.5	0.82
PS	SF	2015	21.7	18.5	925	81	14	5	13.6	24.6	38.2	0.36
PS	ID	2015	24.8	21.2	625	96	0	4	5.5	27.4	33,0	0.17
PS	LT	2016	19.7	15.4	925	89	0	11	34.3	0.9	35.2	0.97
PS	SF	2016	17.2	13.4	1725	88	0	12	10.6	36.8	47.4	0.22
PS	ID	2016	14.7	12.4	1525	100	0	0	2.3	29.3	31.7	0.07
VL	LT	2015	20.6	19.3	975	97	0	3	35.9	0.4	36.3	0.99
VL	SF	2015	24.5	20.3	600	100	0	0	0.0	28.6	28.6	0.00
VL	ID	2015	24.0	21.5	650	100	0	0	9.8	20.1	30.0	0.33
VL	LT	2016	20.1	20.5	1275	45	4	51	39.8	9.5	49.2	0.81
VL	SF	2016	18.5	16.1	800	63	0	38	0.7	26.0	26.7	0.03
VL	ID	2016	19.0	17.8	1800	96	0	4	22.8	39.7	62.5	0.36

[a] Mean tree diameter at breast height (corrected for lost bark)
[b] Mean tree height (measured for 74% of trees)
[c] S% = proportion of spruce, P% = proportion of pine, D% = proportion of deciduous trees

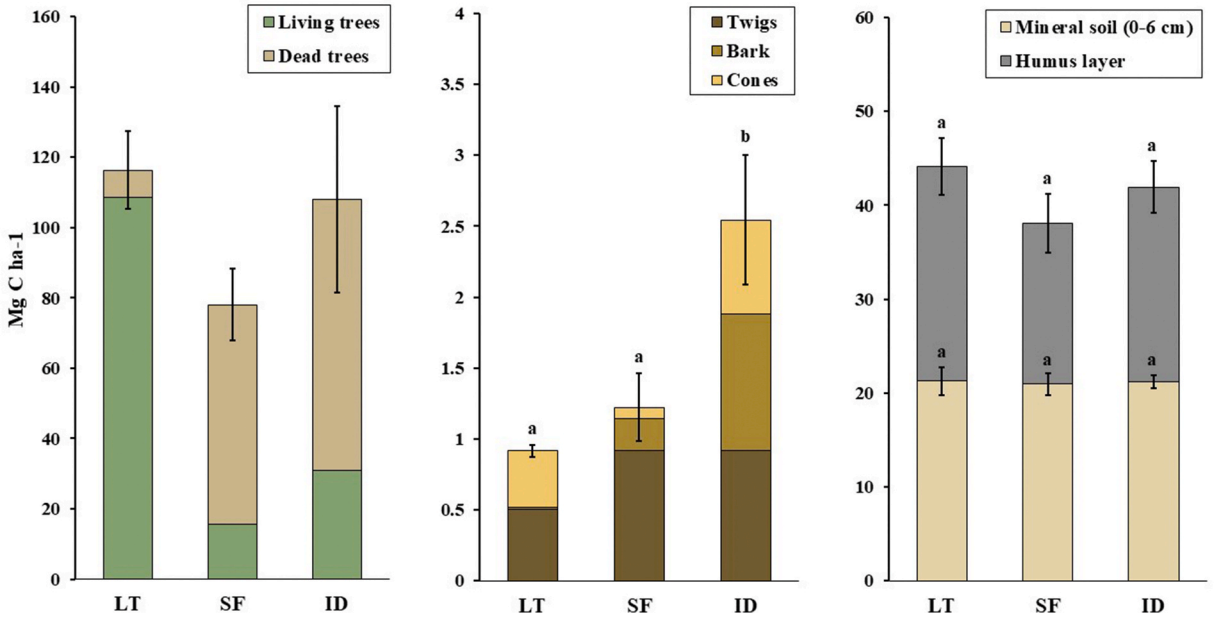

Fig. 1. a) Aboveground tree, b) litter detritus and c) humus layer and mineral soil (0–6 cm depth) mean (±standard error, based on plot mean values) C stocks (Mg C ha^{-1}) of LT (living trees), storm (SF) and *Ips typographus* (ID) disturbed plots. Different letters indicate significant differences in the estimated marginal means of litter detritus or soil C stocks between the plot types (α = 0.05; ANOVA with a mixed model structure and Tukey's post-hoc). Testing of significant differences in litter detritus C stocks between plot types were based on cube-root transformed values.

3.3. C fractions, ergosterol concentration and ECM fungal mycelial growth

No significant differences in humus layer total C concentrations or C_{EXT} between the plot types were found, although their values were lower at SF and ID plots than at LT (Table 2). Removal of an outlier C_{EXT} value in LT, did decrease the LT mean values to 0.98 mg g^{-1} but did not affect the significance of differences between the plot types. Humus layer C_{MB} and ergosterol concentrations were significantly lower at the disturbed plot types in comparison to LT plots. Ergosterol concentrations showed significant interaction between plot type and forest site in the mixed effects model. This was because in Viitalampi, concentrations in the SF (111 mg g^{-1}) and ID (140 mg g^{-1}) plots were significantly lower than those of LT (235 mg g^{-1}) while in Paajasensalo, the concentrations at SF (187 mg g^{-1}) were higher than at ID (141 mg g^{-1}) but still slightly lower than at LT (189 mg g^{-1}), although those differences were not significant. Mean ergosterol concentrations in the ECM in-growth bags were 0.21 for the LT, 0.07 for the SF and 0.05 μg g^{-1} (of sand dry weight) for the ID plots. Mode values of the visual classification of mycelial abundance in the in-growth bags were 1 for the LT, 0 for the SF and 1 for the ID plots. The ECM_{growth} values were thus significantly lower for the disturbed plot types in comparison to LT (Table 2). None of the other variables than ergosterol concentration showed a significant interaction between plot type and forest site. However, magnitude of difference in ECM_{growth} means between LT, SF and ID plots were slightly more distinct in Viitalampi (0.52, 0.17 and 0.16, respectively) than Paajasensalo (0.39, 0.21 and 0.17). Similarly, those of C_{MB} were slightly more distinct in Viitalampi (6.6, 4.6 and 4.9, for LT, SF and ID, respectively) than Paajasensalo (6.7, 5.5 and 5.3).

3.4. Humus layer microbial community composition

At the phylum level over 90% of fungal OTUs were affiliated to three major phyla: Basidiomycota (average proportion 43% of all reads),

Table 2
Mean (±standard error, based on plot mean values) humus layer C fraction and ergosterol concentrations and topsoil ECM fungal abundance and growth index (ECM_{growth}) of LT (living trees), storm (SF) and *Ips typographus* (ID) disturbed plots. All values are calculated per sample dry weight. Different letters indicate significant differences in the estimated marginal means of variables between the plot types (α = 0.05; ANOVA with a mixed model structure and Tukey's post-hoc. Interaction between forest site and plot type was included in the mixed model of ergosterol concentration). Testing of sigificant differences in ECM_{growth} between plot types were based on square-root transformed values.

	Plot type					
	LT		SF		ID	
Total C, mg g^{-1}	461 ± 8.7	a	426 ± 15.4	a	429 ± 12.2	a
Soil extractable C, mg g^{-1}	1.18 ± 0.25	a	0.73 ± 0.03	a	0.80 ± 0.10	a
Microbial biomass C, mg g^{-1}	6.7 ± 0.37	a	5.1 ± 0.28	b	5.1 ± 0.23	b
Ergosterol, µg g-1	212 ± 16.3	a	149 ± 25.1	b	141 ± 7.9	b
ECM_{growth}	0.45 ± 0.042	a	0.19 ± 0.031	b	0.16 ± 0.020	b

Ascomycota (38%) and Mortierellomycota (13%). OTUs representing the most abundant fungal families from total of 87 detected families in all sample types were affiliated to Mortierellaceae (13% of all reads with 25 OTUs), Russulaceae (11% of all reads with 21 OTUs), Cortinariaceae (8% of all reads with 44 OTUs) and Atheliaceae (8% of all reads with 13 OTUs) (data not shown).

From the total 462 fungal and 549 bacterial OTUs, 40 (fungi) and 30 (bacteria) OTUs that best explained the variation in the data and differences between the plot types are shown in the PCA figures (Fig. 2a and b, Supplementary material 2 and 5). Principal components axes 1 and 2 (PC1 and PC2) explained 38% and 28% of the variation in the fungal data respectively, but with only two replicates the significance of axes can only be considered as indicative (Fig. 2a). The majority (88%) of the best fitting 40 fungal representative OTUs shown in the PCA were separated along PC1, indicating higher abundance of their reads in the LT plot types (Fig. 2a, Supplementary material 2). Many OTUs affiliating to common ECM fungal genera such as some *Russula* sp., *Piloderma* sp. and *Cortinarius* sp. were among the most abundant genera in the LT plots but nearly absent in the disturbed plot types (Supplementary material 3). In contrast, OTUs affiliating to *Chaetochytriales* sp., a common ascomycetous, yeast-like group of fungi, and some saprotrophic fungi, e. g. *Mortierella* sp., were slightly more abundant in the disturbed plots (Supplementary material 3). The Venn diagram (Supplementary material 4a) shows that 68% (n = 315) of the fungal representative sequences were observed on all plot types and the LT plot types had more unique OTUs (n = 27) compared to the disturbed plots (n = 17 for SF and n = 12 for ID) and 67% of the unique OTUs at the LT were affiliated to ECM fungal genera.

PC1 and PC2 axes explained 61 and 19% of the variation in the bacterial data respectively, but again with only two replicates the significance of axes can be only be considered as indicative (Fig. 2b, Supplementary material 5). Over 75% of the best fitted bacterial OTUs in the PCA showed specificity towards LT plots (Fig. 2b, Supplementary material 5). Seven OTUs in the PCA, including those affiliating to known genera (e.g. *Acidocella*, *Allohizobium*, *Singulisphaera*, *Burkholderia* and *Occallatibacter*) indicated slightly greater abundance in the LT plots in comparison to disturbed plots and were among the dominant ones (Supplementary material 5 and 6). Five other bacterial OTUs that affiliated to different taxa (e.g., *Rhodoplanes* and *Jatrophihabitans*) showed specificity to the disturbed plots, but they were not among the dominant ones (Supplementary material 5 and 6). However, many common bacterial genera, such as *Bradyrhizobium* and *Acidothermus* had rather similar abundances at each plot type (Supplementary material 6). The Venn diagram of the bacterial OTUs (Supplementary material 4b) shows that the LT plots had almost half less unique bacterial OTUs than the disturbed SF and ID plot types, which had much more shared bacterial OTUs (n = 55) with each other compared to shared OTU amounts with the LT plots (with SF 18 shared OTUs and with ID 8 shared OTUs).

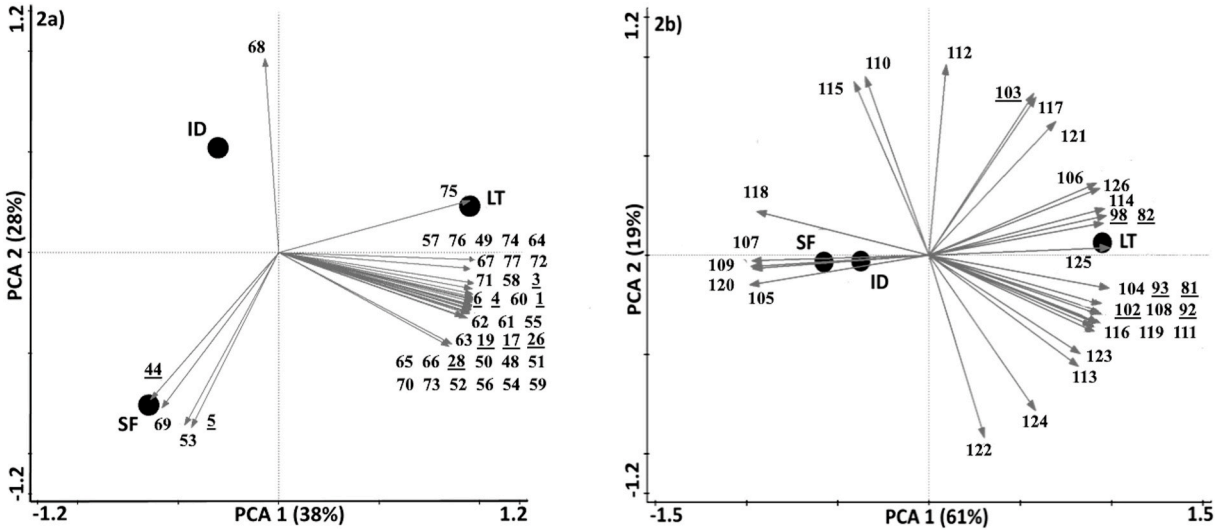

Fig. 2. a) Principal component analysis (PCA) for visualization of the fungal community composition (only the best fitting 40 from the total 462 fungal representative OTUs are shown) and 2b) bacterial community composition (only the best fitting 30 from the total 549 bacterial representative OTUs are shown) between LT (living trees) and disturbed storm-felled (SF) and *Ips typographus* killed (ID) plot types. Numbers refer to the respective OTUs. Of the best fitting 40 fungal OTUs, most abundant (read amount of representative fungal database match from total read amount more than 1% at a plot type) are underlined in the figure. Those were: OTU 1 *Russula decolorans,* OTU 3 *Cortinarius alpinus*, OTU 4 *Piloderma bicolor*, OTU 5 *Chaetothyriales* sp., OTU 6 *Piloderma sphaerosporum*, OTU 17 *Mortierella macrocystis*, OTU 19 *Russula rhodopus*, OTU 26 *Cortinarius* sp., OTU 28 *Tretomyces lutescens*, OTU 44 *Sebacinales* sp. Of the best fitting 30 bacterial OTUs, most abundant (read amount of database match from total read amounts more than 1% at a plot type) ones were: OTU 81 WD260 uncultured Proteobacteria, OTU 82 *Burkholderia* (including also genera *Caballeronia* and *Paraburkholderia*), OTU 92 *Occallatibacter,* OTU 93 uncultured Methylacidiphilaceae, OTU 98 *Singulisphaera*, OTU 102 *Acidocella*, OTU 103 *Allorhizobium* (including also genera *Neorhizobium*, *Pararhizobium* and *Rhizobium)* All of the fitted OTUs can be found in Supplementary material 2 and 5.

4. Discussion

Our first objective was to examine the effects of storm and *I. typographus* induced tree mortality on aboveground tree biomass and necromass, litter detritus (twigs, bark and cones), humus layer and topsoil C stocks. While any difference in tree C stocks between the three plot types would reflect the continued growth of the living trees, decay of the dead trees as well as any difference in biomass between the plots before disturbance, the much greater proportion of tree necromass C on the disturbed plot types is obviously related to storm and *I. typographus* induced tree mortality. The estimated 19% (SF; storm-felled trees) and 7% (ID; *I. typographus* killed trees) decrease in aboveground necromass C however indicated no drastic change in aboveground tree C stocks at the disturbed plots six years (storm) and circa two (*I. typographus*) years after the disturbances.

Our results generally supported the hypothesis that while litter detritus C stocks would be greater at the disturbed plots in comparison to those at the undisturbed (LT) plots, humus layer and top mineral SOC stocks would not differ between plot types. However, only the litter detritus C stocks on the ID plot types were greater than at LT, which could partly be because litter from dead trees at the SF plots was probably more decomposed than at ID. Also, it could relate to differing litterfall inputs from trees killed by storm and *I. typographus*. Bark from beetle killed trees can detach quite fast due to the galleries excavated under bark (Lieutier et al., 2016), whereas more bark seemed to have remained on the dead trees at the SF plots even though *I. typographus* had colonized some of them in 2011–2012. In unmanaged forests where there is no removal of dead trees, for example after bark beetle disturbance, litterfall can remain high for a relatively long time period (Kopáček et al., 2015). The higher or at least similar litter detritus C stocks on both of the disturbed plots in comparison to the LT plots can therefore be expected to persist for some time as the bark, twigs and branches from the dead tree continue to fall to forest floor.

Accelerated decomposition stimulated by increased soil temperature after wind and harvest disturbance has been shown to drive a decrease in SOC stocks in a study in the European Alps (Mayer et al., 2017). Previous research at our study sites, however, indicated little difference in soil temperature or heterotrophic soil surface CO_2 effluxes seven (storm) and circa three (beetle) years after tree mortality (Kosunen et al., 2019). However, for example greatly increased fresh needle litterfall (Kopáček et al., 2015) with a favorable quality for decomposition (Morehouse et al., 2008; Sariyildiz et al., 2008) may have created more notable C losses during or soon after the disturbances. Nevertheless, the similarity in humus layer and mineral soil C stocks between plot types indicated that decomposition of the humus layer and mineral soil organic matter had not been greatly affected by disturbance at the time of the study. The relatively high litter detritus C stocks at the disturbed plots might help to sustain forest floor C stocks for longer than if the dead trees had been harvested.

Secondly, we investigated how the two types of disturbance affected humus layer C fractions and microbial community composition. We had hypothesized that ECM_{growth} and humus layer C_{EXT}, C_{MB} as well as ergosterol (fungal biomass) concentrations would be lowest in the disturbed SF and ID plots (seven and three–four years after tree mortality, respectively), most distinctly at the SF plots. We also expected the microbiological differences between the plot types to be reflected in the differences in bacterial and fungal communities indicated by DNA sequencing. Our results showed that ECM_{growth}, C_{MB} and fungal biomass indeed were lower at the disturbed plots than at LT but C_{EXT} did not significantly differ. Between the disturbed SF and ID plots, magnitude of each variable was however similar.

The lower ECM_{growth} at the disturbed plots was likely due to the death of most host trees, which usually leads to a decreased belowground allocation of photosynthates and a reduction in ECM fungal mycelia (Högberg and Högberg, 2002; Högberg et al., 2001). Since ECM mycelium comprise circa 30–40% of C_{MB} in coniferous forests (Högberg et al., 2010; Högberg and Högberg, 2002), the lower C_{MB} at SF and ID plots was probably mostly due to the decrease in ECM fungi. Although our DNA sequencing results can only be considered directional because of the low number of replicates, they were consistent enough to also indicate a lower abundance and a slightly lower diversity of ECM fungi at the disturbed plots. In coniferous boreal forests, the response of ECM fungal diversity to harvest disturbance has been shown to relate to the amount of retained trees (Sterkenburg et al., 2019), and the recovery of ECM fungal biomass production to occur within a few decades after harvest and tree regeneration (Wallander et al., 2010). The remaining living mature trees and/or proximity of undisturbed forest at our SF and ID plots indeed seemed to have been sufficient to maintain several ECM fungal species typical of old-growth forests (e.g. *Russula* sp.) and would also be expected to fasten the recovery of ECM fungal abundance. Considering forest C sink recovery, recovery of the ECM fungi would be crucial, as they not only increase tree seedling survival and growth (Vaario et al., 2009), but also are very important in immobilizing and storing C to soil (Adamczyk et al., 2019; Clemmensen et al., 2013; Averill et al., 2014).

In addition to the changes in belowground allocation of photosynthates, soil microbial community composition and DOC concentrations would be affected by fluctuations in litter input and its decomposition after tree-killing disturbance (Kaňa et al., 2015; Štursová et al., 2014; Trahan et al., 2015). That C_{EXT} concentrations were not significantly lower at SF and ID plots in comparison to LT, was possibly due to decomposition of litter detritus and organic matter and the belowground C allocation from the remaining living trees, seedlings and ground vegetation partly compensating for decreased supply of C_{EXT} from mature living trees.

The lower fungal biomass at the disturbed plots in comparison to the LT plots likely owed to the decrease in ECM fungi and did not indicate such increases of saprotrophic fungal biomass that would have compensated fully for the losses in ECM fungi. However, the DNA sequencing results indicated that certain decomposing (e.g. *Chaetothyriales* sp. and *Mortierella* sp.) fungi had benefited from the disturbance and resulting tree necromass and litter detritus at the storm and *I. typographus* affected plots. The significant interaction between plot type and forest site (Paajasensalo and Viitalampi) for the humus layer fungal biomass (ergosterol concentration) was due to the difference in values between LT and the disturbed plot types being more distinct and differences between the SF and ID plots being opposite at Viitalampi in comparison to Paajasensalo. This may be related to the narrower difference in proportions of living to total tree basal area, and consequently also in ECM_{growth}, between the LT and SF plots at Paajasensalo in comparison to Viitalampi.

Bacterial biomass was not separately quantified in our study, however, the lower microbial biomass (of similar level as fungal biomass) at the disturbed plot types indicated no great changes in bacterial biomass due to the disturbances. Although the DNA results indicated a community shift and slightly greater bacterial diversity at the disturbed plots, abundance of the most dominant bacterial OTUs did not seem to greatly differ between the plot types. However, some dominant bacterial OTUs showed higher specificity and abundance in LT plots. One of these was *Occallatibacter,* belonging to the N-suppressed genera of Acidobacteriaceae (Subgroup 1). Three other of the bacterial OTUs that matched to known taxa and affiliated to Proteobacteria of the *Acidocella* and *Burkholderia* genera and Rhizobiaceae family have been shown to be typical bacterial taxa in Swedish Norway spruce forests (Haas et al., 2018). *Burkholderia* was more abundant in root than in soil samples (Haas et al., 2018) and dominant in the ectomycorrhizosphere of soil--*Quercus petraea-Scleroderma citrinum* continuum (Uroz et al., 2013), suggesting their colonization with tree-associated mycorrhizal fungi. Some bacterial OTUs showed higher specificity towards disturbed plots, but their low general abundance in our limited data prevents any further interpretation. Soil bacterial community composition has previously been shown to be relatively little affected by storm and bark beetle

disturbance (Ferrenberg et al., 2014; Šimonovičová et al., 2019) and the magnitude of changes relate to the proportion of remaining living trees (Mikkelson et al., 2017), which might have helped to stabilize the bacterial community in our disturbed plots.

Tree mortality resulting from storm disturbance is immediate and commonly includes soil disturbance while an *I. typographus* outbreak is more gradual, dispersed over time and often leads to patchier tree mortality. Tree mortality in our study area indeed varied from individuals to stand-level, suggesting that there may be considerable variation and differences in soil C stocks and fractions and microbial communities in the disturbed areas. However, our results indicated no pronounced differences in the effects of storm and *I. typographus* disturbance on those variables. This might be to some extent explained by our sampling strategy and the rather similar mean basal area of dead trees on our SF and ID disturbed study plots. In addition, although the difference in the nature of storm and *I. typographus* disturbance would be expected to be reflected in the development of the intensity and timing of the effects on C cycling, over time, the effects might become similar. However, as the two disturbances in our study occurred at different times, their impact on C cycling would be at different stages of development, and therefore it is difficult to attribute any similarity or difference between the SF and ID plot types to disturbance type only.

5. Conclusions

In spite of the more gradual development and intensity of *I. typographus* outbreaks in comparison to storm events, in our study the two disturbance types appeared to have rather similar effects on humus layer C and microbiology, which may be related to the difference in the time since the disturbance (study conducted five to seven years after storm and one to four years after *I. typographus* disturbance). The shift from living tree biomass to necromass C stocks was reflected in greater litter detritus C stocks at the beetle plots. However, no differences in SOC stocks or humus layer extractable C concentrations between the plot types were shown. Humus layer microbial biomass C and ergosterol concentrations and ECM fungal abundance were lower on disturbed plots in comparison to undisturbed ones. DNA sequencing indicated that the disturbed plots had a lower abundance and slighty lower diversity of ECM fungi, but a slightly higher abundance of some saprotrophic fungi, whereas no pronounced differences in the most abundant bacteria were indicated between the plot types. More distinct or differing effects of storm and *I. typographus* disturbance on soil C stocks and fractions as well as microbial community may be expected when looking at differing spatial scales and because of variation in the pattern of tree mortality. We investigated the disturbance-mediated effects after only some years following the disturbance, but to understand the full impact of such disturbances on forest functioning and C balance, long-term monitoring studies will be required.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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